

UNIFORMED SERVICES UNIVERSITY OF HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

FEBRUARY 2005

APPROVAL SHEET

Title of Thesis: "Field and Laboratory Application of a Gas Chromatograph Low
Thermal Mass Resistively Heated Column System in Detecting
Traditional and Non-Traditional Chemical Warfare Agents using Solid
Phase Micro-Extraction"

Name of Candidate: LCDR David R. Koch
Master of Science in Public Health
Department of Preventive Medicine and Biometrics

Thesis and Abstract Approval

Chairman: Col(ret) Robert J. Fitz, MSPH

Date

Research Advisor: CDR Gary Hook, PhD, MPH

Date

Thomas E. Johnson, PhD

Date

De Chang Chen, PhD

Date

LtCol Michael J. Roy, MD, MPH

Date

Report Documentation Page		Form Approved OMB No. 0704-0188
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.		
1. REPORT DATE FEB 2005	2. REPORT TYPE	3. DATES COVERED -
4. TITLE AND SUBTITLE Field and Laboratory Application of a Gas Chromatograph Low Thermal Mass Resistively Heated Column System in Detecting Traditional and Non-Traditional Chemical Warfare Agents using Solid Phase Micro-Extraction		5a. CONTRACT NUMBER
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Uniformed Services University of the Health Sciences (USUHS),4301 Jones Bridge Road,Bethesda,MD,20814-4799		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited		
13. SUPPLEMENTARY NOTES The original document contains color images.		
14. ABSTRACT Emergency response units (ERUs) need a chemical sampling and analysis method that will allow rapid, and accurate on-scene detection and identification of dangerous chemicals. Fast gas chromatography (FGC) using a low thermal mass, resistively heated, toroidal (LTM-RHT) column, along with solid phase microextraction (SPME) was used as an analytical method to sample and detect frank contamination of traditional chemical warfare agents (CWAs), CWA precursors, and non-traditional CWAs (i.e. narcotics). The research results were used to evaluate the ability of the LTM-RHT column to effectively separate and identify a complex mixture of CWAs in the laboratory and in the field, and to evaluate the ability of the LTM-RHT column coupled to a mass spectrometer to rapidly separate and identify a complex mixture of narcotics. Various sampling times and column temperature ramping rates were used to evaluate peak resolution (R), column efficiency (CE) and/or peak retention time (RT). The results of the CWA sampling and analysis indicate the LTM-RHT column assemblies could be used by an (ERU) to effectively analyze and detect frank chemical warfare agent contaminated clothing up to 75% faster than a column that is heated using a standard ramping rate for an air bath oven (ABO). The decrease in analysis time, to include system cool-down time, will allow for a potentially significant increase in the number of samples that can be analyzed within a given time period. The data of the CWA precursors shows that identification with sufficient peak separation of volatile analytes via FGC is obtainable. The work with non-traditional CWAs demonstrated that FGC can significantly reduce analysis retention time while maintaining adequate peak resolution, and column efficiency.		
15. SUBJECT TERMS		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 59	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

**Field and Laboratory Application of a Gas Chromatograph Low
Thermal Mass Resistively Heated Column System in Detecting
Traditional and Non-Traditional Chemical Warfare Agents using Solid
Phase Micro-Extraction**

Beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of Health Sciences from any damage, which may arise from such copyright violations.

David R. Koch
LCDR, MSC, U.S. Navy
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Thesis: “Field and Laboratory Application of a Gas Chromatograph Low Thermal Mass Resistively Heated Column System in Detecting Traditional and Non-Traditional Chemical Warfare Agents using Solid Phase Micro-Extraction”

Author: LCDR David R. Koch
Master of Science in Public Health

Thesis Directed by: CDR Gary Hook, PhD
Assistant Professor
Department of Preventive Medicine and Biometrics

Emergency response units (ERUs) need a chemical sampling and analysis method that will allow rapid, and accurate on-scene detection and identification of dangerous chemicals. Fast gas chromatography (FGC) using a low thermal mass, resistively heated, toroidal (LTM-RHT) column, along with solid phase microextraction (SPME) was used as an analytical method to sample and detect frank contamination of traditional chemical warfare agents (CWAs), CWA precursors, and non-traditional CWAs (i.e. narcotics). The research results were used to evaluate the ability of the LTM-RHT column to effectively separate and identify a complex mixture of CWAs in the laboratory and in the

field, and to evaluate the ability of the LTM-RHT column coupled to a mass spectrometer to rapidly separate and identify a complex mixture of narcotics. Various sampling times and column temperature ramping rates were used to evaluate peak resolution (R), column efficiency (CE) and/or peak retention time (RT).

The results of the CWA sampling and analysis indicate the LTM-RHT column assemblies could be used by an (ERU) to effectively analyze and detect frank chemical warfare agent contaminated clothing up to 75% faster than a column that is heated using a standard ramping rate for an air bath oven (ABO). The decrease in analysis time, to include system cool-down time, will allow for a potentially significant increase in the number of samples that can be analyzed within a given time period.

The data of the CWA precursors shows that identification with sufficient peak separation of volatile analytes via FGC is obtainable. The work with non-traditional CWAs demonstrated that FGC can significantly reduce analysis retention time while maintaining adequate peak resolution, and column efficiency.

FIELD AND LABORATORY APPLICATION OF A GAS CHROMATOGRAPH LOW
THERMAL MASS RESISTIVELY HEATED COLUMN SYSTEM IN DETECTING
TRADITIONAL AND NON-TRADITIONAL CHEMICAL WARFARE AGENTS
USING SOLID PHASE MICRO-EXTRACTION

BY

LCDR DAVID R. KOCH

Thesis submitted to the Faculty of the Department of Preventive Medicine and
Biometrics Graduate Program of the Uniformed Services University of the Health
Sciences in partial fulfillment of the requirement for the degree of the Master of Science
in Public Health, 2005

DEDICATION

To my wife, Badia, and my children Sofia and Mehdi for your patience and support, and sacrifices you've had to make while I attended school, and all other times I was away from home. I dedicate this thesis to you.

To my mom and dad, Ann and Marvin Koch, thank you for believing in me, providing guidance while allowing me to cut my own path.

ACKNOWLEDGMENT

I am grateful to the U.S. Marine Corps Systems Command for providing funding for this research, the National Institute for Drug Abuse (NIDA) for providing some the essential materials, Dr. Carmela Jackson-Lepage and the Defence Research and Development Canada-Suffield for providing the facilities to conduct some of this research. I would like to thank the Veterinary Science Staff at the Armed Forces Radiobiological Research Institute (AFRRI), LtCol Hildabrand and SFC Heflin, and Uniformed Services University of Health Sciences' (USUHS) Controlled Substances Board members, Ms. Andrea Sorrells and Mr. David Travis, and Mr. Henri Weems for obtaining and maintaining the narcotics required for this research.

I would like to thank all the members of my committee for their time and effort in assisting me in completing this program. I would like to thank Col(ret) Robert Fitz and Dr. Johnson for providing some the best courses in the Environmental and Occupational Health program. I especially want to thank CDR Gary Hook and CDR Philip Smith, and Dr. Brian Eckinrode of the Federal Bureau of Investigation (FBI) for their outstanding training, direction and support in making this research possible.

TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

Statement of Problem.....	1
Background.....	1
Research Goal.....	4
Research Question.....	5
Specific Aims.....	5

CHAPTER TWO: LITERATURE REVIEW

Analytes.....	7
Nerve and Blister Agents and Precursors.....	7
Narcotics.....	9
Sampling with Solid Phase Micro-Extraction.....	10
Fast Gas Chromatography.....	12

CHAPTER THREE: METHODS

Materials.....	18
Laboratory Sampling and Analysis of Nerve and Blister Agents.....	19
Field Sampling and Analysis of Nerve and Blister Agents.....	22
Laboratory Sampling and Analysis of Narcotics.....	23
Laboratory Sampling and Analysis of Nerve Agent Precursors.....	27

CHAPTER FOUR: RESULTS AND DATA ANALYSIS

Laboratory Analysis Results of Nerve and Blister Agents.....	28
Field Analysis Results of Nerve and Blister Agents.....	33
Laboratory Analysis Results of Nerve Agent Precursors.....	34

Laboratory Analysis Results of Narcotics.....	35
CHAPTER FIVE: CONCLUSIONS, LIMITATIONS, AND	
RECOMMENDATIONS	
Conclusions.....	40
Limitations and Recommendations.....	41
BIBLIOGRAPHY.....	43

LIST OF FIGURES AND TABLES

Figures

Figure 1: Resistively Heated Toroidal Column.....	17
Figure 2: Viking 573 GC-MS with RVM LTM-RHT Column Module.....	21
Figure 3: Viking 572 GC-MS with RVM LTM-RHT Column Module.....	23
Figure 4: Agilent 6890 GC-MS with RVM LTM-RHT Column Module.....	26
Figure 5: GC-MS chromatograms of chemical warfare agents and degradation products using a Viking 573 GC-MS and RVM LTM-RHT DB1-HT column and a PDMS-DVB SPME sampling fiber.....	29
Figure 6: GC-MS chromatograms of chemical warfare agents and degradation products using a Viking 573 GC-MS and RVM LTM-RHT DB1-HT column and a PDMS SPME sampling fiber.....	30
Figure 7: GC-MS chromatograms of chemical warfare agents and degradation products using a Viking 572 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber (Sampling time: 10 min).....	32
Figure 8: GC-MS chromatograms of chemical warfare agents using a Viking 572 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber (Sampling time: 5 min).....	33
Figure 9: GC-MS chromatograms of chemical warfare agent precursors using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 second and temperature ramping rate of 20°C/min from 40-250°C).....	34
Figure 10: GC-MS chromatograms of chemical warfare agent precursors using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 second and temperature ramping rate of 80°C/min from 40-250°C).....	35
Figure 11: GC-MS chromatograms of 5 µg/mL narcotic mixture using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber (Sampling time: 1min. and temperature ramping rate of 20°C/min from 40-250°C).....	36
Figure 12: GC-MS chromatograms of 5 µg/mL narcotic mixture using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 minute and temperature ramping rate of 60°C/min from 40-250°C).....	36

Figure 13: GC-MS chromatograms of 5 µg/mL narcotic mixture using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 minute and temperature ramping rate of 120°C/min from 40-250°C).....37

Tables

Table 1: Characteristics of CWA Detectors.....	3
Table 2: Physical Properties of Selected Nerve and Blister Agents.....	9
Table 3: Physical Properties of Selected Narcotics.....	10
Table 4: Retention Time Comparative Analysis.....	38
Table 5: Column Efficiency Comparative Analysis.....	39
Table 6: Peak Resolution Comparative Analysis.....	39

CHAPTER ONE: INTRODUCTION

Statement of the Problem

Civilian and/or military Emergency Response Units (ERU) are responsible for providing initial post-incident consequence management and assisting in minimizing the effects of the contaminants in the event of an accidental or intentional release of chemicals. The release of highly dangerous chemicals must be mitigated quickly to protect the public and the environment. An ERU, such as the Marine Corps' Chemical and Biological Incident Response Force (CBIRF), must be able to conduct a rapid assessment of contaminated sites, and extract casualties. Rapid detection and analysis capabilities are essential for an ERU to be able to efficiently triage and stabilize individuals for further treatment, however such capabilities are predominantly limited to the laboratory. An ERU must be able to quickly identify sources of frank contamination, thereby providing timely information to on-scene commanders, who will be able to effectively minimize the spread of the contamination, and designate safe areas for decontamination and medical support teams [1].

Background

Chemicals used against military and civilian populations have been documented since 400 BC. The past century has seen nerve agents, blister agents, and narcotics used as lethal or incapacitating weapons. New hazardous chemicals are developed each year [2, 3]. Only a relative few are tested as to their acute or chronic effects on humans and the environment. Depending on the concentration, mode of dispersion, environmental persistence, route of entry and transport, and site of deposition, any chemical could

adversely effect on an individual. The Department of Defense (DoD) as well as other federal agencies, and state and local response teams are constantly searching for alternative sampling and analysis methods for rapid detection of chemicals in the environment. Several relatively simple and fast methods are available for identifying the presence of chemicals, particularly chemical warfare agents (CWA) in field settings. Those methods include the military's M-8 and M-9 chemical detection papers and the M272 and M256A1 chemical agent detector kits, and commercially available chemical detection tubes. Field portable equipment utilizing Surface Acoustic Wave (SAW), Infrared Spectroscopy (IRS), Flame Ionization Detection (FID), Photometric Ionization Detection (PID), and Ion Mobility Spectrometry (IMS) are also available. The capabilities and limitations of these detection methods are summarized in Table 1 [4]. These methods are highly portable and are easy to use [2, 5, 6]. However, many of these methods are not suitable for use in identifying an unknown field sample, detecting and distinguishing a specific contaminant from a mixture of chemicals or detecting a chemical at low concentrations. Some methods are not able to perform sampling and or analysis unless the chemical is in a specific phase (e.g. vapor vs. liquid). Often, additional manipulations, such as solvent extraction, have to be performed before final analysis can be conducted. The results of these methods may provide only qualitative data, and can be influenced by interfering substances and environmental conditions, which may result in false positives [4].

ERUs need a chemical sampling and analysis method that will allow rapid, and accurate on-scene detection and identification of dangerous chemicals. An ideal detection/identification method will be fast, easy to use, field portable, and will provide

the ability to accurately identify unknown analytes in complex chemical mixtures.

Table 1: Characteristics of CWA Detectors

Detector	Agent	Specificity and Sensitivity	Response Factor Issues	Time	Agent Phase
PID	CWA and Toxic Industrial Chemicals (TIC)	Low/High	Humidity, Mixtures ₄	<3 min	Vapor
Colorimetric					
M8 paper	CWA _{1,2}	Med/Low	-	<1min	Aerosol
M9 Paper	CWA _{1,2}	Low/Low	-	<1min	Aerosol
M272 Kit	CWA/TIC	High/Med	-	<10min	Agent in water
M256A1 Kit	CWA	High/Low	-	<15min	Vapor
Detector Tubes	CWA/TIC	High/Low	Humidity	<10min	Vapor
SAW	CWA/TIC	High/Med	Humidity, Mixtures ₄	<3 min	Vapor
IR Spectroscopy	CWA/TIC	Med/Med	Humidity, Mixtures ₄	<3 min	Aerosol, Liquid, Solid
FID	CWA/TIC	High/Med	-	<3 min	Vapor
IMS	CWA ₃	High/Med	-	<3 min	Vapor
Standard GC-MS ₅	CWA/TIC	High/High	-	<25min	Vapor/Liquid
LTM ₆ GC-MS	CWA/TIC	High/High	-	<3 min	Vapor/Liquid

1. Nerve 2. Blister 3. Choking 4. Mixtures refer to a methods difficulty in detecting and identifying a single contaminant from a mixture of chemicals 5. Gas chromatography-mass spectrometry 6. Low thermal mass

To limit personnel exposure and reduce the spread of contamination, rapid sampling combined with analysis at the site of contamination is desired. Analysis by a method such as gas chromatography-mass spectrometry (GC-MS) is needed to confirm the identities of chemical compounds actually present with a high degree of certainty. There is a greater level of confidence in the identification of a substance with GC-MS, because it provides orthogonal data (i.e. retention time, peak area, and mass of the ions present), which is not available with the other detection systems [7]. A current portable GC-MS sampling and analysis method suitable for field use is a 16 kg man-portable GC-MS system that samples and analyzes gas phase contaminants at the site of contamination [8]. Recent research has focused on improving field-sampling and analysis methods.

Solid phase micro extraction (SPME) and Fast Gas Chromatography (FGC) are

two techniques which provide the potential for rapid sampling and analysis in both laboratory and field settings of vapor, gas, and liquid samples [9-11]. Standard sampling of chemical agents for GC-MS analysis usually involves absorbing/adsorbing media for field sampling, followed by extensive sample preparation procedures that are performed in a laboratory. Analysis is typically conducted by injecting a liquid or gas sample into the injection port of a GC-MS. The sample is run through a capillary column, which is heated in an air bath oven (ABO), where the chemical(s) are separated. The chemicals exit the column and enter the Mass Spectrometer where they are ionized and detected.

SPME is an alternative strategy proposed for sampling of volatile and semi-volatile chemicals in field settings. Analysis is performed by insertion of the SPME sampling fiber into the heated injector of a field-portable GC-MS system operated at a fixed location near the site of contamination for analysis [12-19]. FGC employing the use of a Low Thermal Mass Resistively Heated Toroidal (LTM-RHT) Column is a new technique to reduce analysis time [20]. SPME sampling and FGC analysis methods will potentially allow for the quick, on-scene sampling and identification of chemicals, and increase the number of samples that can be analyzed within a given time period [21-24].

Research Goal

The goal of this research was to determine the efficacy of sampling and analysis instruments that can be fielded to detect traditional CWAs, CWA precursors, and non-traditional CWAs (i.e. narcotics). It is through this research that sampling and analysis methods can be developed that are both effective and efficient for detecting chemical agents, that will provide a method for an ERU to obtain rapid, high quality data, and that parallel the governments efforts in finding new and innovative ways to protect its citizens

[25].

Research Question

Can a rapidly heated, low thermal mass, resistively heated, toroidal gas chromatography column run at a standard temperature program, perform as well as, or better when run at a fast temperature program in analyzing traditional and non-traditional CWAs, without losing resolution?

Specific Aims

The specific aims of this research were to:

(1) Qualitatively determine if a rapidly heated LTM-RHT column combined with a GC-MS can effectively separate and analyze a mixture of traditional CWAs (e.g. 1-methylethyl methyl phosphonoflouridate (Sarin - GB), 1,2,2-trimethylpropyl methylphosphonoflouridate (Soman - GD), Cyclohexyl methylphosphonoflouridate (Cyclosarin - GF), S-(2-(bis(1-methylethyl) amino)diethylamino)ethyl)-O-ethylmethylphosphonothioate (V gas - VX), and Bis(chloroethyl) sulfide (HD – Distilled Mustard)) in a laboratory [26].

(2) Qualitatively determine if a rapidly heated LTM-RHT column combined with a GC-MS can effectively separate and analyze a mixture of CWAs (e.g. GB, GD, GF, VX, and HD), in a field setting.

(3) Simulate a crude field sample (cotton swab) of a mixture of nerve agent precursors (e.g. Dimethyl methylphosphonate (DMMP), Diethyl methylphosphonate (DEMP), Diisopropyl methylphosphonate (DIMP), Diethyl ethylphosphonate (DEEP)) and determine if a rapidly heated LTM-RHT Column combined with a GC-MS can effectively separate and qualitatively identify individual components [27].

(4) Qualitatively determine if a rapidly heated LTM-RHT Column combined with a GC-MS can effectively separate and analyze a mixture of non-traditional CWAs (i.e. Fentanyl, Alfentanil, Remifentanil, Sufentanil, and Carfentanil) in a laboratory.

(5) Quantitatively determine a maximum temperature ramping rate at which the LTM-RHT Column combined with a GC-MS can separate and analyze a mixture of non-traditional CWAs (i.e. Fentanyl, Alfentanil, Remifentanil, Sufentanil, and Carfentanil), without reduction in resolution compared to similar analysis using a standard temperature ramping rate that is used by columns that are heated in an ABO.

CHAPTER TWO: LITERATURE REVIEW

Analytes

The DoD and other agencies involved with national defense, such as the Federal Bureau of Investigation, have been developing methods to rapidly detect chemicals. The chemicals of interest are those that could be, or have been used to develop CWAs, or used as weapons of war or acts of terrorism. These agents include potent narcotics, nerve and blister CWAs, and their precursors. One of the primary goals is for an ERU to be able to quickly obtain high quality data in order to detect and identify these agents in the field. This information can then be forwarded to those responsible for directing the response actions required to mitigate further exposure of personnel and environmental contamination.

The nerve agents used in this research were chosen because of their use in recent world events, and because of their similar physical properties and physiological effects. A mustard agent was also part of the CWAs to be studied, because of its use in world events. The precursors used in this research were chosen due to their availability and chemical similarity to nerve agents such as Sarin and Soman [26-28].

The narcotics were chosen based upon their similar physical properties and physiological effect. Carfentanil, in particular, has been used as a non-traditional offensive chemical weapon [29, 30].

Nerve and Blister Agents and Precursors

Nerve agents are organophosphorous cholinesterase inhibitors and have similar mechanisms of toxicity. When an organophosphorous nerve agent binds to acetylcholinesterase, acetylcholine accumulates at cholinergic receptor sites and

continues to stimulate affected organs. The effects of nerve agent exposure include miosis, rhino rhea, bronchoconstriction, loss of consciousness, muscle twitching, seizures, fatigue, weakness, flaccidity, apnea, cyanosis, hypertension, bradycardia, and death. Nerve agents are similar to the Opioids in that they both affect the central nervous system and have been used to either kill or incapacitate people. Nerve and blister CWAs are volatile to semi-volatile chemicals. Their persistence in the environment varies indirectly with their volatility. For example, VX, HD and, to a lesser degree, GF are semi-volatile and are more environmentally persistent than the more volatile G-agents such as GB and GD.

VX and the G-agents are classified as nerve agents and are the most toxic of the traditional CWAs. VX is an odorless, amber colored liquid. GB is both odorless and colorless. GD, and GF are both colorless, but GD has a fruity odor [3, 29-31].

HD is a persistent CWA. It is an oily substance that is yellowish-brown in color, and has a slight garlic odor. HD is an alkylating agent that produces blisters on the skin, mucous membranes and in the lungs and is therefore classified as a vesicant or blister agent. While not as toxic as nerve agents, HD may produce systemic effects in the bone marrow, gastrointestinal tract, and in the central nervous system. It is also considered to be a carcinogen. Death from HD exposure is usually a result of respiratory failure [2, 28, 30]. Physical characteristics of these CWAs are provided in Table 2 [26-28, 30, 32].

Table 2: Physical Properties of Selected Nerve and Blister Agents

Name	VX	GB	GD	GF	HD
Formula	C ₁₁ H ₂₆ NO ₇ PS	C ₄ H ₁₀ FO ₂ P	C ₇ H ₁₆ FO ₂ P	C ₇ H ₁₄ FO ₂ P	C ₄ H ₈ Cl ₂ S
Molecular Weight	267.4	140.1	182.2	108.16	159.08
Melting Point °C	-39	-56	-42	-12	13-14
Boiling Point °C	298	158	198	239	215-217
Density g/ml@ °C	1.008@20	1.102@20	1.022@25	1.13@20	1.27@20
Vapor Pressure mmHg@ °C	0.0007@20	2.10@20	0.40@25	0.044@20	0.11@25
Volatility mg/m ³	10.5	22,000	3,900	438	920
Vapor Density	9.2	4.9	6.3	6.2	5.5
Water Solubility g/L	30	Miscible	21@20°C	3.7	0.92
Henry's Law Constant	3.5x10 ⁻⁹	5.4x10 ⁻⁷	4.6x10 ⁻⁶	*	2.1x10 ⁻⁵
Log Kow	2.09	0.299	1.824	*	1.37
LD ₅₀ g/70kg	0.01	1.7	0.35	0.35	1.4

* Data not available

Narcotics

Narcotics, such as Morphine, Fentanyl and Fentanyl's congeners Sufentanil, Alfentanil, Lofentanil, and Carfentanil, are classified as opioids. They are predominantly used during perioperative periods as analgesic anesthetic adjuncts, to augment general anesthesia, and reduce postoperative pain. Fentanyl and its congeners are synthetic opioids. They are lipophilic and can readily cross the blood-brain barrier [33].

The more potent opioids could effectively be used as a lethal weapon at high enough concentrations. In comparison to Morphine, Alfentanil, Fentanyl, Sufentanil, and Carfentanil are 15, 100, 1000, and 7500 times more potent, respectively [33, 34]. Opioids produce analgesia, drowsiness, confusion, euphoria, and alter respiratory, cardiovascular, gastrointestinal, and neuroendocrine functions. The use of opioids results in a decrease in respiratory and heart rate, and a reduction in blood pressure. Common effects include

nausea, vomiting, confusion, and muscle rigidity. At higher doses, they can impair ventilation, and cause neuro-excitation and seizures. Intracranial pressure may occur if ventilation is not controlled. Pinpoint pupils and coma categorize an overdose, which may result in death from respiratory failure [30, 33]. Physical characteristics of the above narcotics, considered semi-volatile chemicals, are provided in Table 3 [3, 35, 36].

Table 3: Physical Properties of Selected Narcotics

Name	Fentanyl	Alfentanil	Sufentanil	Lofentanil	Remifentanil	Carfentanil
Formula	$C_{22}H_{28}N_7O$	$C_{21}H_{32}N_6O_3$	$C_{22}H_{38}N_2O_2S$	$C_{25}H_{32}N_2O_3$	$C_{20}H_{28}N_2O_5$	$C_{24}H_{30}N_2O_3$
Molecular Weight	336.48	416.52	386.55	408.54	376.43	394.51
LD ₅₀ mg/kg skin	3.1	47.5	17.9	0.066	*	3.4
ED ₅₀ mg/kg skin	0.011	0.044	0.007	0.0059	*	0.0034
Melting Point °C	148-150	135-140	133-140	*	*	*
Solubility in water g/L 20°C	27	130	25	*	*	*
Log KoW	4.09	2.21	4.02	*	17.9	*

* data not available

Sampling with Solid Phase Micro-Extraction

Traditional sampling methods have required the collection of bulk samples for analysis of analytes in a solid matrix (i.e. soil or water). The use of sampling pumps and various collection media (i.e. sorbent tubes and filter cassettes) are required to sample for airborne or vapor phase analytes. Preparation and analysis of these traditional samples often requires complex analytical instrumentation, an extensive power supply, and the use of hazardous materials other than the primary analytes, in order to prepare the samples for analysis. Analysis of organic compounds is usually performed using a GC and a suitable detector (i.e. Flame Ionization Detector). The limitations of such a sampling/analysis combination include length of sampling and analysis time, the need for

additional chemicals for sample preparation, and in some instances a lack of sensitivity (i.e. a high limit of detection) [37, 38]. Depending on the sample media, the environmental concentration of the contaminant, and the sensitivity of the detector, large sample volumes may be required in order to obtain a detectable concentration level. Larger sample requirements increase both sampling time and the potential for personal exposure.

SPME uses an injectable fiber, coated with a sorbent material, in order to sample volatile and semi-volatile chemicals. SPME has been used to directly sample air and water, as well as the headspace above a variety of sample matrices including water, soil and other solids [5, 6, 8, 12, 15, 19, 39-41]. SPME combines sampling, extraction, concentration and sample preparation into a single step [15]. SPME is a solvent-free process that extracts organic compounds and concentrates them on a thin, fused, silica fiber coated with a stationary phase. SPME fibers come in two classes (absorptive or adsorptive fibers), and five different types. Absorption is a non-competitive process that does not result in the complete extraction of an analyte from a sample matrix unless the concentration of the analyte is extremely low and has a very high affinity for binding to a specific fiber. Adsorption is a competitive process where analytes compete for pore binding sites on the surface of the SPME fiber. The size of the pore space enhances the sensitivity for analytes based on their molecular size.

The absorptive class of fibers consists of two types of fiber coatings, Polydimethylsiloxane (PDMS) and Polyacrylate (PA). The PDMS is used for non-polar analytes while the PA is better suited for polar analytes [42].

The adsorptive class of fibers consists of a solid polymer particle, either Divinyl

Benzene (DVB) or Carboxen, mixed into a PDMS or Carbowax phase. There are three types in common use, a Carbowax-DVB phase coated fiber, used for sampling polar analytes, a PDMS-DVB fiber, and a PDMS-Carboxen fiber, both the latter coatings are used for sampling non-polar analytes [42].

There are three extraction methods for SPME, direct, headspace, and membrane. In the direct mode, an aqueous matrix is sampled by direct contact with the SPME fiber. In the headspace mode, the fiber is placed into the space above the sample and the analytes, as they partition into the headspace from the sample, are absorbed/adsorbed to the fiber coating. In the third method, a membrane is placed between the sample and the fiber in order to protect the fiber from high levels of contaminants [42, 43]. Injecting the exposed fiber into the heated inlet of a GC or other analytical instrument causes desorption of the analyte.

The benefits of SPME include a reduced sample time, and a reduction in preparatory steps, equipment, and solvents used. SPME, combined with GC-MS, will allow for complete on-site exposure assessment of chemicals of interest [12, 15, 37, 38, 41, 44].

Fast Gas Chromatography

Analysis of CWAs using GC provides for effective separation of volatile to semi-volatile analytes [13, 14, 17, 45]. When GC is coupled with a mass spectrometer, it allows for definitive identification of an analyte [7]. However, analysis of an analyte using a conventional GC and capillary column typically takes from 10 to 30 minutes and in some instances more time may be required depending upon the size of the oven, the length of the column, the environmental conditions in which the analysis is being

performed, and the initial and final oven temperature settings for each run [15, 20, 44, 46-49]. One of the limiting factors in reducing analysis time is the design of the column used to separate the analytes. Standard column design requires a GC with an ABO to heat the column. More energy is required to heat a column in an ABO than to heat an LTM-RHT column due to the ABO's larger thermal mass. The larger thermal mass of the ABO also takes more time to heat up and cool down, increasing the length of the analysis. The length of time between successive runs is dependent upon the temperature ramp settings, equipment cool-down time, and the time needed to reset the equipment. Ramping rates for standard air bath ovens can reach 120 °C/minute, with the average rate being 20-30 °C/minute [44, 49, 50]. It may take 7 to 11 minutes for a standard oven to cool down from 270 °C to 40 °C, depending on the external environmental conditions [20].

Oven heating can be responsible for various adverse effects that impact the analysis including degradation of the analyte and/or column stationary phase. Column degradation can lead to exposed active sites on the column and excessive peak tailing. Peak tailing occurs when an analyte remains stationary too long and spreads out within the column. ABO heating may also cause uneven heating of the column and lead to poorly developed peaks [20, 46].

Alternative FGC methods to reduce analysis time have been reduction of the length and/or diameter of the column, increased carrier gas flow rate, increased temperature ramping rates, use of a thinner column stationary phase, and use of a resistively heated column [20, 44, 46, 49, 51-53]. Use of these various methods is dependent upon the chemical(s) to be analyzed, and their properties such as boiling points, retention times, and the resolution between eluting peaks. Use of one or a

combination of these techniques may result in overlapping peaks, fronting, column overload, and/or poor analysis of trace analytes [44, 49, 51]. One of the first FGC methods employed a capillary column placed in a metal tube or a column wrapped with a metal wire. In both instances, voltage was applied to the conductive material in order to heat and control the column temperature. This design, commonly referred to as a resistively heated column (RHC), allows the column to be heated and cooled much more rapidly than is possible in an ABO [20, 44, 46, 47, 49-51, 54]. Ramping rates with resistively heated column techniques have ranged from 30 to 1200 °C/min, and owing to the low thermal mass inherent to such a system, and the size of the column, cooling from 300 to 50 °C in 30 seconds. Overall analysis time can potentially be reduced 10-fold, shortening a 30 minute analysis to 2.5 minutes in many instances [20, 44, 46, 49, 51, 53].

One of the primary goals for developing a system that can allow for an increase in ramping speed is to reduce the analysis time without compromising resolution or separation efficiency. In order to obtain optimum separation only those peaks/analytes of interest should be separated [50, 51]. The use of shorter narrow bore columns, faster carrier gas velocities, and faster column heating can speed up analysis time. However, the use of shorter columns may not be suitable for chemical mixes that have a narrow range of boiling points, are very volatile, or that are close to their optimum resolution [18, 20, 44, 49, 50].

In one study, the effects of column heating and column length were compared to analysis time. A standard column (100 m), narrow bore column (40 m), RHC (10 m) and RHC (6 m) were used. The analysis times for each were 2 hours, 45 minutes, 35 minutes and less than 5 minutes, respectively. The combination of the RHC and column length

significantly reduced the analysis time. However, shorter columns resulted in poorer resolution and separation efficiency when compared to standard columns (typically 30 m in length) [50, 53]. Resolution and separation efficiency may also decrease with RHC as a result of increased ramping rates (i.e. 200-600°C/min as compared to 20-40°C/min) [20, 37, 44, 46, 48, 50, 55]. In the case of the heated metal sheath covered column, if the sheath did not make contact along the entire length of the column, uneven thermal heating occurred [20, 44, 49]. Initial research indicates that the use of a toroid assembly results in greater temperature ramping rates and rapid cool down times. The packaging of the column minimizes exposed surface areas of the components, provides electrical insulation without restricting thermal conduction, and improves temperature uniformity, thereby reducing the chance for uneven heating to occur [20].

Even with these limitations, FGC does provide advantages in addition to the previously mentioned rapid analysis. RHCs require less power and take up less space as compared to standard columns heated in an ABO [37, 46, 47]. So far, they have been used with some success to analyze volatile and semi-volatile analytes such as petrochemicals, organophosphates, and polycyclic aromatic compounds [20, 48].

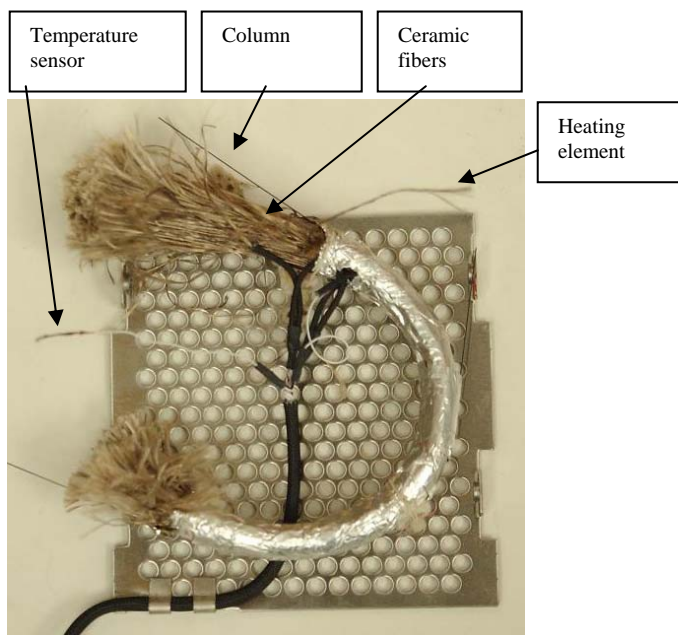
One of the newest RHCs on the market is a toroid assembly. A toroid is a coil of insulated or enameled wire wound on a donut-shaped form made of powdered iron. A toroid is often used as an inductor in electronic circuits, especially at low frequencies where comparatively large inductances are necessary. A toroid assembly has the advantage of providing more inductance for a given number of turns than a solenoid with a core of the same material and similar size. This makes it possible to construct high-inductance coils of reasonable physical size and mass. Toroidal coils of a given

inductance can also carry more current than solenoidal coils of similar size, because larger-diameter wires can be used, and the total amount of wire is less, reducing the resistance [56]. In this case, the use of the toroid structure significantly reduces the spatial volume of the column.

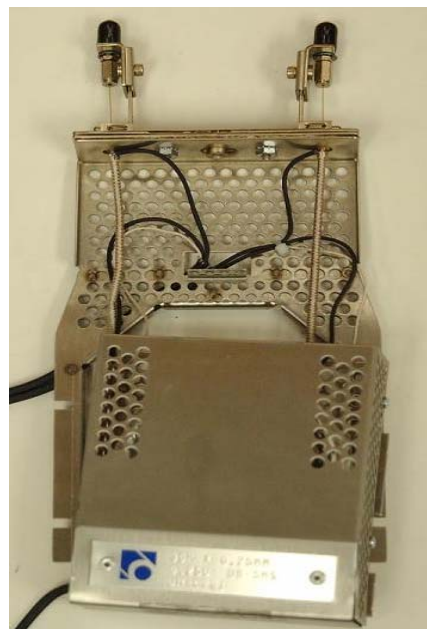
The resistively heated toroidal (RHT) column has a low thermal mass (LTM) and consists of an open tubular, bonded-liquid phase type column wrapped in aluminum foil. Precise temperature control is made possible through a resistive heating element (nickel wire), a temperature sensor (platinum wire), and a ceramic insulation sleeve covering the heating element, which are in contact with the GC column inside the aluminum wrapping as shown in Figure 1 [46, 47].

As with the RHT Columns previously mentioned, initial research indicates the use of the toroid assembly results in improved heat exchange, which allows for greater temperature ramping ranges, and faster cool-down time. The packaging of the column minimizes exposed surface areas of the components, provides electrical insulation without restricting thermal conduction, and improves temperature uniformity [20].

Figure 1: Resistively Heated Toroidal Column



(A) Cut LTM-RHT column exposing temperature sensor, heating element and ceramic fibers



(B) LTM-RHT column and cage

CHAPTER THREE: METHODS

Materials

The individual components of the nerve and blister CWA agent mixture sampled were obtained from Defence Research and Development Canada (DRDC) – Suffield (Medicine Hat, Alberta, Canada). All chemical handling was performed in a chemical laboratory hood where the hood effluent was scrubbed through a charcoal filter. A stock solution of each compound was prepared from neat material freshly distilled at the Canadian National Single Small Scale Facility using a Kugelrohr apparatus. Purities were verified by proton nuclear magnetic resonance (^1H NMR) and GC-MS to be >99% for each of the G-series compounds, >98% for VX, and >97% for HD.

The individual components of the narcotic mixture sampled were obtained from a variety of sources. Carfentanil Hydrochloride (200 mg) was obtained from the National Institute for Drug Abuse, through the Research Triangle Institute, (Raleigh, NC). Sufentanil Citrate (50 $\mu\text{g/mL}$) was obtained from Elkins-Sinn Inc, (Cherry Hill, NJ), Alfentanil Hydrochloride (500 mg/mL), Fentanyl Citrate (50mg/mL), and Remifentanil Hydrochloride (1mg/vial) were obtained from Abbott Laboratories (North Chicago, IL).

The individual components of the nerve agent precursor mixture sampled were obtained from two sources. DIMP (96%) was obtained from Alfa Aesar (Wardhill, Ma). DMMP (97%), DEMP (97%), and DEEP (98%) were obtained from Aldrich-Fluka, (Steinheim, Switzerland).

The SPME fiber and holder used were obtained from Supelco (Bellefonte, PA). The fiber coatings used were polydimethylsiloxane (PDMS), and PDMS-DVB. Two

different fiber coatings were used to evaluate the possible variability in affinity for the CWAs. SPME fiber selection for CWA sampling (i.e. PDMS and PDMS-DVB) was based on research results using GB, VX, and HD [19, 40, 57, 58]. SPME selection for narcotic sampling (i.e. PDMS-DVB) was based on the results from fiber optimization research using Sufentanil in human plasma [59]. Sampling using SPME was consistent throughout the research. Blank runs were completed at least once daily before use of any fibers for sampling to ensure no carryover of analytes from previous extractions. SPME fibers were conditioned prior to use following the manufacturer's recommendations.

Laboratory Sampling and Analysis of Nerve and Blister Agents

From each stock solution, 40 μ L of each neat chemical was injected with a Hamilton gastight macro-volume syringe (Hamilton, Reno, NV) into a 15 mL silanized vial (i.e. the mixed agent vial), fitted with a PTFE-lined silicon septum, open screw top closure (Supelco Bellefonte, PA). Samples were created at room temperature (23°C). From the mixed agent vial, 1 μ L was injected into another 15 mL silanized vial containing a piece of a standard green, cotton-fabric, military undershirt material. This method represented an ERU encountering an individual, contaminated with an unknown liquid agent, in which the clothing is removed and sampled.

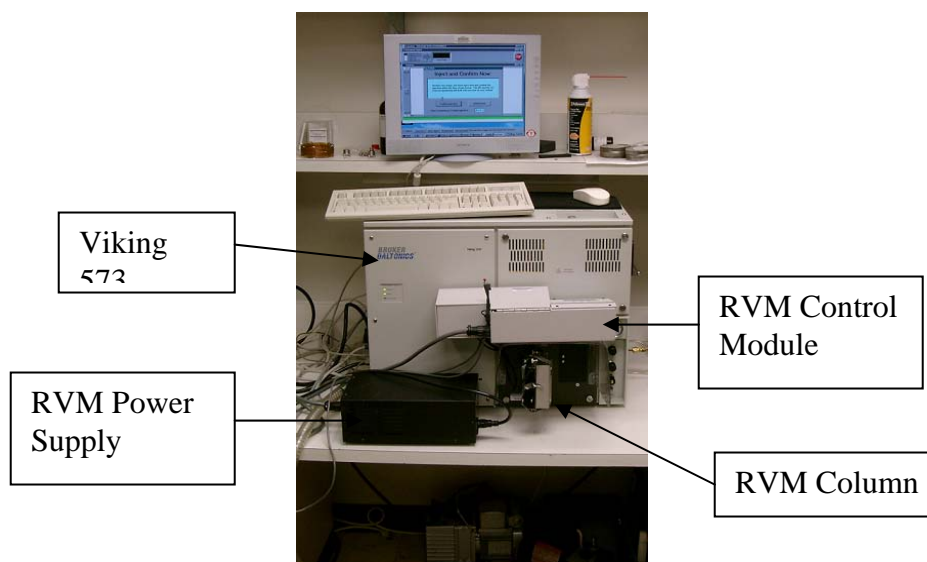
SPME sampling was accomplished by piercing the septum of the fabric-containing vial. After the septum was pierced with the SPME fiber assembly, the fiber was extended into the vial for a defined extraction period of <1.0, 1.0, 10.0, 30.0 or 60.0 seconds. Differing lengths of sampling time was employed in order to find the optimum time that would provide the best peak shape for each analyte. The sampling times for each run were randomly selected. At the end of an extraction period, the SPME fiber was

retracted into its protective sheath, removed from the vial and immediately introduced into the heated GC injection port. The fiber was then quickly lowered into the midrange region of the heated injection port liner (0.75 mm ID deactivated glass, Supelco) and GC-MS analysis commenced. Desorption of the SPME fiber samples was accomplished in the splitless injection mode. Due to the restricted access to these CWAs and the short time available for sampling and analysis, only qualitative results were obtained. At least three samples were desired for each sample time. However, due to safety concerns and time constraints, only one sample per set of variables (i.e. sample time, and SPME fiber coating), could be obtained and analyzed.

GC-MS lab sampling and analyses were completed using a field potable Viking 573 GC-MS instrument (Bruker Daltronics, Billerica, MA), Figure 2. The MS portion of the instrument was based on a Hewlett Packard 5973 ion source and monolithic quadrupole mass filter, and Chemstation data system. Electron Impact (EI) (70 eV) ionization was used and mass spectra were collected over a range of 10-250 mass-to-charge ratios. Hydrogen, initially set at 17 psi with a linear velocity of 100 cm/s, was used as the carrier gas. A 2-stage Edwards G/E oil-based rotary roughing pump was used to maintain pressure within the MSD. A 2 minute solvent delay was employed during each analysis. The injection port and transfer lines were maintained at 250 °C, the source and the quadrupole were set at 230 °C and 106 °C respectively. Multiple blank runs were conducted with the GC ABO temperature program set for a standard ramp in order to obtain ABO cool-down time data. For the blank runs the ABO was initially at 40 °C, held for 5 seconds and then ramped to 250 °C at 20 °C/min. While employing the LTM-RHT column, the GC ABO was maintained at an isothermal temperature of 250 °C.

The LTM column assembly used was built by RVM Scientific (Santa Barbara, CA), and contained a 30 m DB1-HT column, having a 0.25 mm I.D. and a 0.25 μ m film thickness (J&W Scientific, Folsom CA.). In addition to the analytical column assembly, the GC column transfer lines leaving the LTM-RHT column assembly were also resistively heated on the external side of the statically heated air bath oven, and were temperature-controlled. A stand-alone module provided column-heating control with a keypad for temperature programming. The control module and a power supply (110-230 V AC, 100 W) are depicted in Figure 2. The externally mounted LTM-RHT column temperature parameters were as follows: 40 °C initial temperature was held for 5 seconds, then ramping to 100 °C at 80 °C/min, then up to 115 °C at 20 °C/min, followed by ramping to 250 °C at 200 °C/min.

Figure 2: Viking 573 GC-MS with RVM LTM-RHT Column Module



Field Sampling and Analysis of Nerve and Blister Agents

DRDC created a stock solution of 400 ng/ μL GB, GD, GF, and HD by diluting the neat agents in methylene chloride. An agent mixture with a 5.0 mg/m^3 concentration of each agent was created by injecting 50 μL of each stock solution into a Tedlar bag (SKC Inc, Eighty –Four PA) containing 4.0 L of air. The contents were allowed to equilibrate at room temperature for 30 minutes. This method represented an ERU sampling the vapors of an unknown liquid agent encountered in the field.

SPME sampling, using a PDMS-DVB fiber, was accomplished by piercing the septum of the Tedlar bag and extending the fiber into the bag for an extraction period of 5 minutes. The SPME fiber was retracted into its protective sheath, removed from the bag and immediately introduced into the heated GC injection port. The fiber was then quickly lowered into the midrange region of the heated injection port liner and GC-MS analysis commenced. Desorption of the SPME fiber samples was accomplished in the splitless injection mode.

GC-MS field analyses were completed as previously discussed in the laboratory analysis, with the exception that a field portable Viking 572 GC-MS instrument (Bruker Daltronics, Billerica, MA) was used, Figure 3. The MS portion of the instrument was based on a Hewlett Packard 5972 ion source and monolithic quadrupole mass filter, and Chemstation data system. EI (70 eV) ionization was used and mass spectra were collected over a range of 35-350 mass-to-charge ratios. The injection port and transfer lines were maintained at 250 °C and 225 °C respectively. The source and the quadrupole were set at 270 °C and 180 °C respectively. The GC oven was maintained at an isothermal temperature of 200 °C.

The LTM-RHT column assembly (30 m DB5-MS column) and method was the same as that stated in the laboratory analysis with the exception of the temperature parameters. The externally mounted LTM-RHT column temperature parameters were as follows: 40 °C initial temperature was held for 5 seconds, then ramping to 180 °C at 120 °C/min.

Figure 3: Viking 572 GC-MS with RVM LTM-RHT Column Module



Lab Sampling and Analysis of Narcotics

All narcotics had to be in solution prior to sampling. The Fentanyl, Alfentanil, and Sufentanil were purchased in solution. Remifentanil and Carfentanil were obtained in a solid state. 1 mg of Remifentanil and 5 mg of Carfentanil were separately dissolved in 1 mL of deionized water. One milliliter of Fentanyl, Alfentanil, and Sufentanil, and half a milliliter of Remifentanil and Carfentanil were placed into a separate 4 mL silanized vial using a 1 mL Hamilton gastight macro-volume syringe (Hamilton, Reno, NV). 2 mL of a 10% Sodium Chloride solution were added to each vial containing a single narcotic. Ammonium Hydroxide (25% in water - Fluka-Aldrich) was added to each vial to cause the solution to be basic. Additional diluted solutions of Carfentanil, Alfentanil, and

Remifentanyl were created from the original stock solutions so that all of the analyte solutions had a concentration of 25 $\mu\text{g}/\text{mL}$. Finally, 0.5 mL of each of the narcotic analyte solutions was placed into a 4 mL silanized vial resulting in a 5 $\mu\text{g}/\text{mL}$ concentration of each analyte.

SPME sampling was accomplished by piercing the septum of the 4 mL glass vial with PTFE-lined silicone septum fitted in an open screw top closure. After the septum was pierced with the PDMS-DVB fiber assembly, the fiber was extended into the solution for a defined extraction period of 1 minute. At the end of an extraction period, the SPME fiber was retracted into its protective sheath, removed from the vial, rinsed with deionized water to remove any salts, then with methanol (Fisher Scientific, Fairlawn NJ) to remove the water [52]. The fiber was allowed to air dry for 30 seconds, and then introduced into the heated GC injection port. The fiber was then quickly lowered into the midrange region of the heated injection port liner and GC-MS analysis commenced. Desorption of the SPME fiber samples was accomplished in the splitless injection mode.

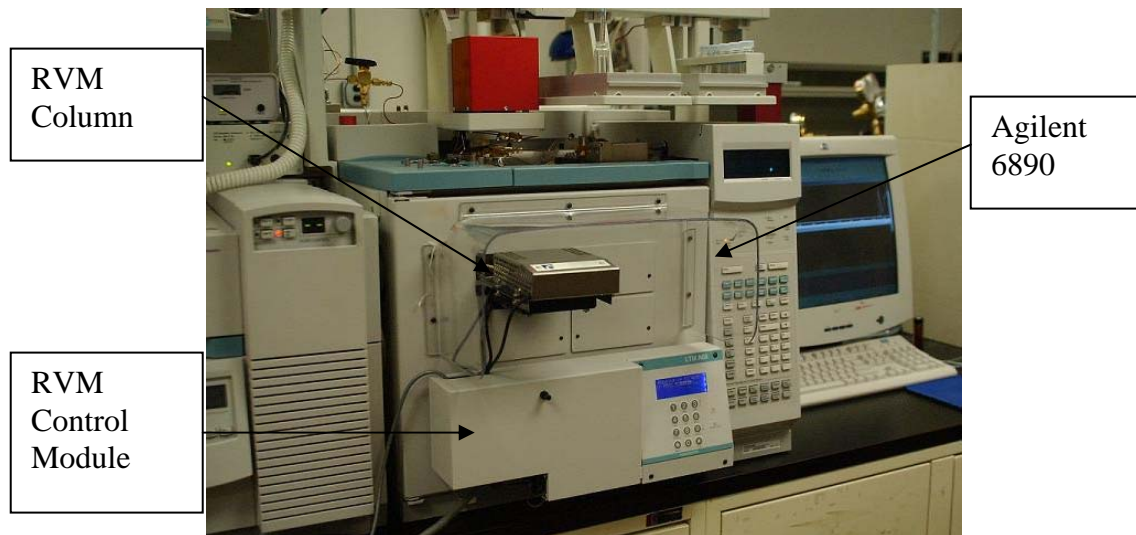
GC-MS lab analyses were completed using an Agilent 6890/5973 GC-MS instrument (Agilent Technologies, Billerica, MA). EI (70 eV) ionization was used and mass spectra were collected over a range of 100-310 mass-to-charge ratios. Helium, initially set at 34 psi with a linear velocity of 115 cm/s, was used as the carrier gas. Total flow at the inlet was set at 7.9 L/min with a flow of 4.2 L/min in the column. The injection port and transfer lines were maintained at 225 °C, the source and the quadrupole were set at 230 °C and 150 °C respectively. The GC oven was maintained at an isothermal temperature of 250 °C.

The LTM-RHT column assembly used was built by RVM Scientific (Santa Barbara, CA) and contained a 15 m DB1-MS column, having a 0.25 mm I.D. and a 0.25 μ m film thickness (J&W Scientific, Folsom Calif.). To obtain a chromatogram representative of a standard ABO temperature ramping configuration, the externally mounted LTM-RHT column temperature parameters were as follows: 40 °C initial temperature, then ramping to 250°C at 20 °C/min and held for 2.5 minutes. Total run time was 13 minutes, which included a 10-minute solvent delay. To determine a maximum temperature ramping rate in which retention time was reduced without a significant reduction in resolution, chromatograms were obtained employing two different temperature ramping rates. The parameters for the first rapid temperature ramping rate were set as follows: 40 °C initial temperature, then ramping to 250°C at 60 °C/min and held for 3.5 minutes. A solvent delay was employed therefore analysis was started at 4.2 minutes into each GC-MS run. Total run time was 7 minutes. The parameters for the second temperature ramping rate were as follows: 40 °C initial temperature, then ramping to 250°C at 120 °C/min and held for 5.25 minutes. A solvent delay was not employed. Total run time was 7 minutes. The results of the slow and fast temperature ramping rates were used for comparative analysis. A GC-MS and LTM-RHT column assembly with a 30 m column is shown in Figure 4.

Performance of the standard and fast temperature ramping rates using the resistively heated column were quantitatively evaluated by comparing differences in column efficiency (CE), resolution (R) between peaks, and retention times (RT). CE is the relationship between a peak's RT and its width. R is the measure of separation between two peaks, which depends upon the width of the peaks and their corresponding

retention times. RT is a measure of how long it takes a compound to travel through the column. It is the sum of the time the compound spends in the stationary and mobile phases, and is dependent upon the type and dimensions of the column, column temperature, and carrier gas linear velocity[60, 61]. Two types of peak

Figure 4: Agilent 6890/5973 GC-MS with RVM LTM-RHT Column Module



widths are used in measuring column performance, the base width, and the width halfway up the peak (i.e. full width at half maximum - (Wh)). When calculating CE, the base width (Wb) of the peak is used. The formula for Wb is described in formula (1). CE can be expressed as the number of theoretical plates (N). A greater number of plates results in thinner peaks at their respective RT. The formula for calculating N is described in formula (2). When determining R, the Wh is used. The formula for calculating R is described in formula (3). A resolution of 1.5 represents fully resolved peaks[60, 62]. The minimal statistical significant difference of the CE, RT, and R between the two temperature ramping programs was calculated using the student t-test for each of the three data sets.

$$(1) \quad W_b = 1.699(W_h)$$

$$(2) \quad N = 16(RT / W_b)^2$$

$$(3) \quad 2(RT_{\text{of peak 2}} - RT_{\text{of peak 1}}) / (W_{\text{h of peak 1}} + W_{\text{h of peak 2}})$$

Laboratory Sampling and Analysis of a mixture of CWA Precursors

For sampling and analysis of CWA Precursors (DMMP, DIMP, DEMP and DEEP), 2 μ L of each of the chemicals were injected on to the end of a long stemmed cotton swab that had been broken off and placed into a 4 mL silanized vial. The use of a cotton swab to obtain a field sample has been employed by a military ERU team. The sampling and analysis method was the same as that for the narcotics mixture with the exception of the sampling time, and scan range. Sampling time was 1 second. The mass spectra were collected over a range of 90-170 mass-to-charge ratios. The temperature ramping parameters were changed to simulate a standard and fast temperature ramping programs. For the standard ramping program, the initial temperature was 40 °C. The column temperature was then ramped to 120°C at 20 °C/min. The run time was 3 minutes. For the fast ramping program, the column was initially held at 40 °C, and then ramped to 120°C at 80 °C/min, and held for 2 minutes. Total run time was 3 minutes.

CHAPTER FOUR: RESULTS AND DATA ANALYSIS

Laboratory Analysis Results of Nerve and Blister Agents

Sampling and analysis performance of the SPME fiber coatings and the LTM-RHT column were qualitatively evaluated by comparing the quality of the CWA chromatograms with regard to sampling time, peak height, analyte retention time, and column cool-down time between successive runs.

Results in Figures (5-7) provide GC-MS (EI) total ion chromatograms. In comparing the chromatograms of Figure 5 or 6, the results indicate that the peak height increases in proportion to sampling time. In comparing the chromatograms of Figure 5 and 6, the results indicate that the PDMS-DVB fiber has a greater affinity for the CWAs than the PDMS fiber. The retention time and cool-down time for the chromatograms of Figures 5 or 6, and 7 indicate there is a significant decrease in retention time and cool-down time, without an appreciable loss in resolution.

In the chromatograms provided in figures 5 and 6, in which the sampling times were the same but the sampling media was different, there was a general difference in peak height. Both a fiber's affinity for specific analytes as well as the length of sampling time can affect peak area, height, and resolution of the chromatogram peaks. Peak shape and resolution between the analytes improved as the sampling time was reduced. Notably, the resolution of the diastereomer peaks of GD improved once the sampling time reached 10 seconds.

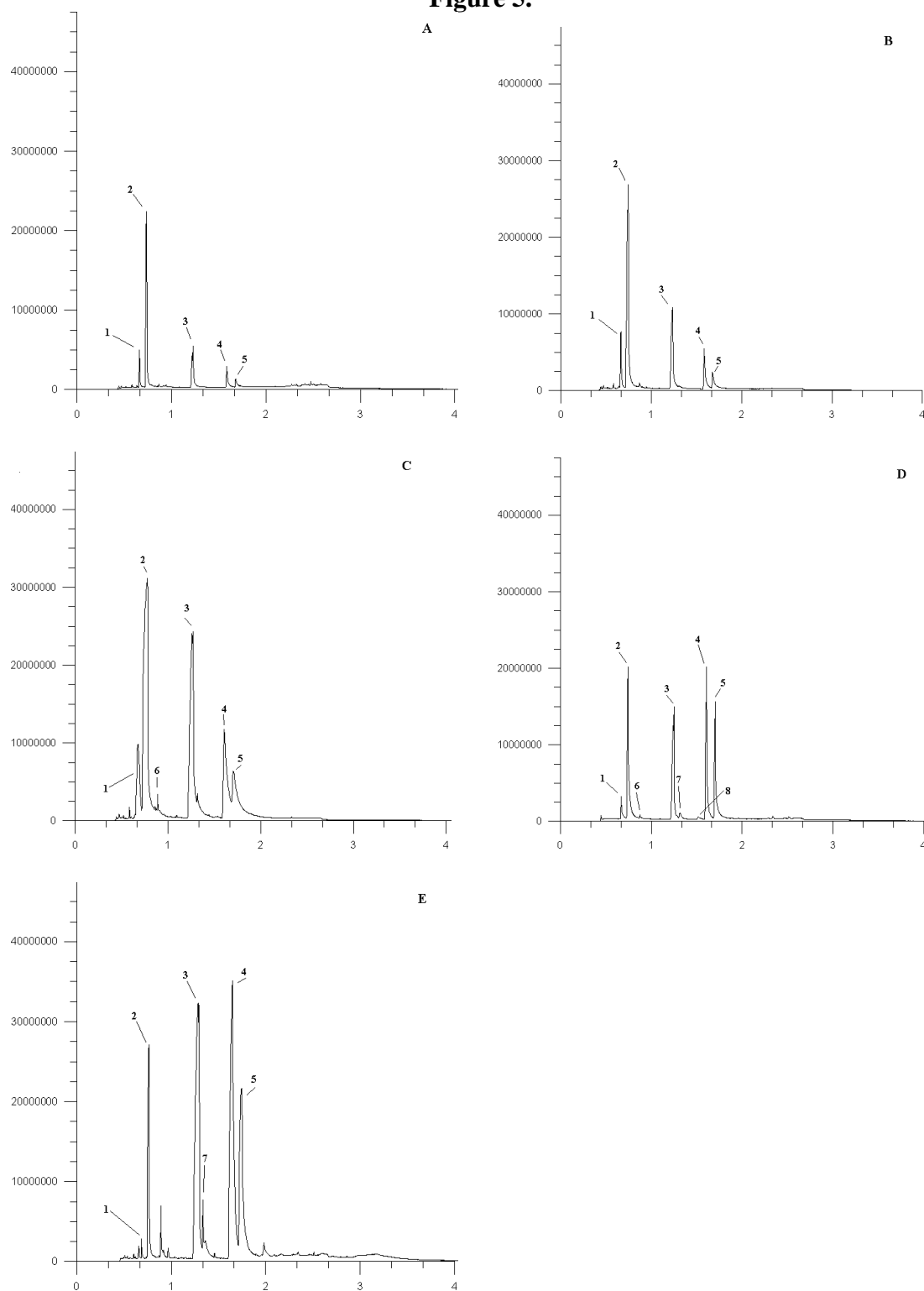
Figure 5.

Figure 5: GC-MS chromatograms of chemical warfare agents and degradation products using a Viking 573 GC-MS and RVM LTM-RHT DB1-HT column and a PDMS-DVB SPME sampling fiber. (Sampling time: A. <1sec, B. 1sec, C. 10sec, D. 30sec, E. 60sec.) (Peaks: 1. Ethyl methylphosphonofluoridate, 2. Sarin, 3. Soman, 4. Distilled Mustard, 5. Cyclohexyl methylphosphonofluoridate, 6. 2-Methylcyclopentyl methylphosphonofluoridate, 7. Diisopropyl methylphosphonate, 8. 2-(Diisopropylamino) ethanethiol.) Column heating ranged from 20-200°C/min.

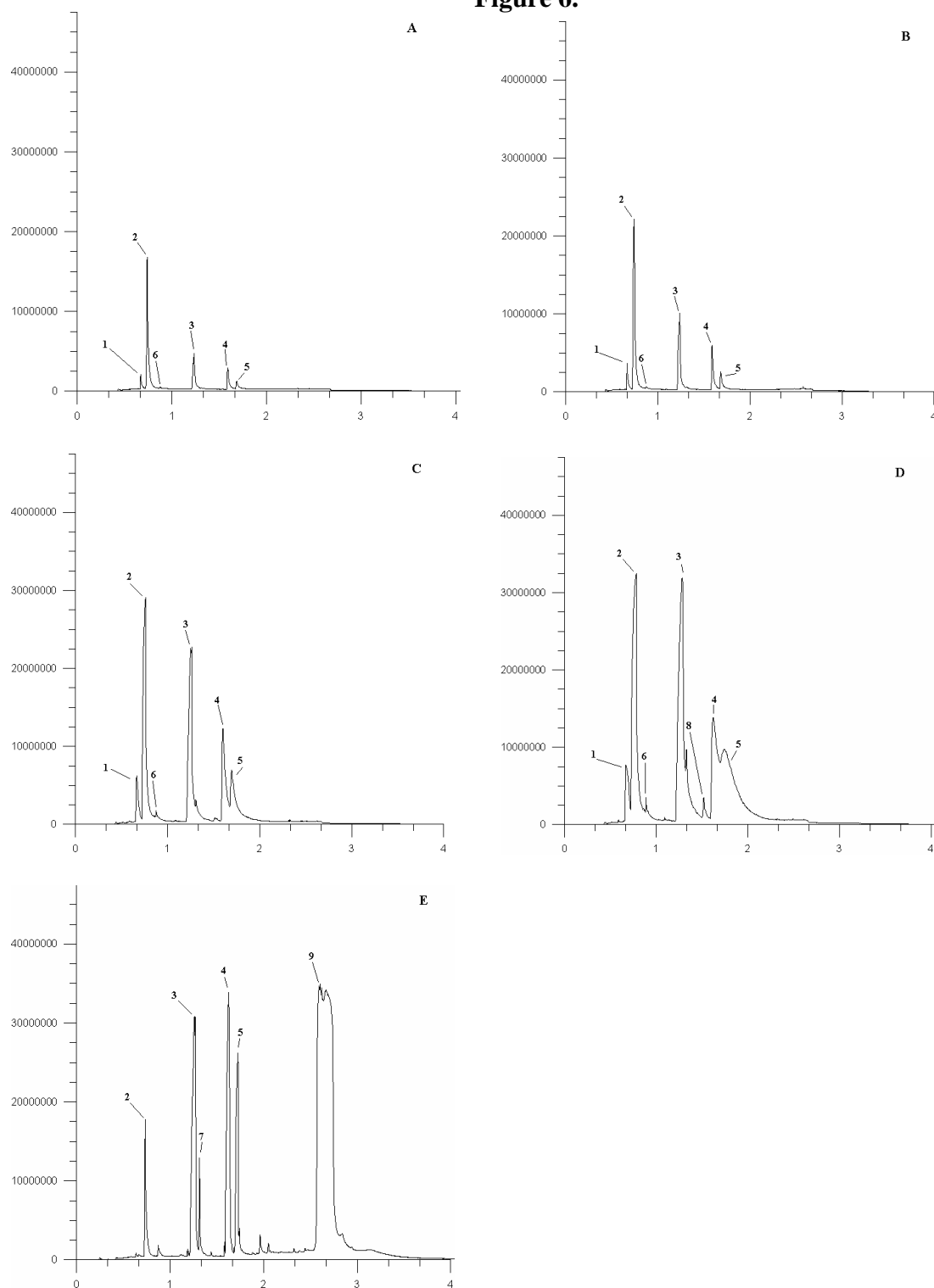
Figure 6.

Figure 6: GC-MS chromatograms of chemical warfare agents and degradation products using a Viking 573 GC-MS and RVM LTM-RHT DB1-HT column and a PDMS SPME sampling fiber.(Sampling time: A. <1sec, B. 1sec, C. 10sec, D. 30sec, E. 60sec.) (Peaks: 1. Ethyl methylphosphonofluoridate, 2. Sarin, 3. Soman, 4. Distilled Mustard, 5. Cyclohexyl methylphosphonofluoridate, 6. 2-Methylcyclopentyl methylphosphonofluoridate, 7. Diisopropyl methylphosphonate, 8. 2-(Diisopropylamino) ethanethiol, 9. VX.) Column heating ranged from 20-200°C/min.

Along with the length of sampling time, the time at which the sample was taken after the initial spiking of the vial with the agent mix may have resulted in a difference in the peak height, area, and identification of analytes between the chromatograms on each figure. For example, the results of chromatogram (6E) were from the first sample and analysis performed, using the PDMS fiber – DB1-HT column assembly, after spiking the cloth in the vial with the agent mix. VX was only identified on this chromatogram. Sampling and analysis of intact VX is extremely difficult [19, 39]. For VX analysis, the sample should be heated to at least 50 °C prior to sampling [39]. However, the presence of chemical warfare agents can be ascertained by identifying the existence of their respective degradation products [13, 14, 19, 28, 40]. Several degradation products were identified for GB, GD, and VX. Retention times and mass spectra library search matches were obtained for Diisopropyl methylphosphonate, Ethyl methylphosphonate, Cyclohexyl methylphosphonofluoridate, Methylcyclopentyl methyl phosphonofluoridate, and 2-(Diisopropylamino)ethanethiol [28, 37]. The presence of VX degradation products may explain why VX was difficult to identify. As the sampling time increased more of the various degradation products were identified.

Figure 7 is a chromatogram obtained from the sampling and analysis of a 5-agent mixture. The parameter for the LTM-RHT column programmed temperature ramping was as follows: held the initial temperature of 35 °C for one minute, then ramped to 150°C at 20 °C/min and held for 6.25 minutes. The retention times for GB, GD, HD, and GF on this chromatogram were 3.6, 6.4, 8, and 8.5 minutes respectively.

In comparison, the average retention times for the same chemical warfare agents, using the LTM-RHT column assembly listed in Figures 5 and 6, were 0.85, 1.47, 1.8,

1.87 minutes respectively. The LTM-RHT column used for Figure 7 reached its maximum-programmed temperature within 6.75 minutes, whereas the columns listed in Figures 5, 6 and 7 reached their maximum-programmed temperature within 2.175 minutes. Both analysis methods (Figures 5-7) maintained adequate separation between the various peaks for unambiguous identification of the chemical warfare agents.

Figure 7:

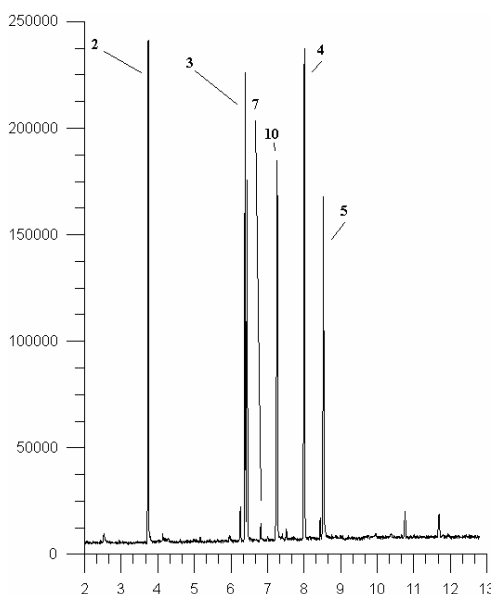


Figure 7: GC-MS chromatogram of chemical warfare agents and degradation products using a Viking 572 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber.(Sampling time: 10 min.) (Peaks: 2. Sarin, 3. Soman, 4. Distilled Mustard, 5. Cyclohexyl methylphosphonofluoridate, 7. Diisopropyl methylphosphonate, 10. Ethydimethylphosphoramidocyanide – Tabun [26])

To reduce analysis time, two important factors are retention time and oven-column recycling time. ABOs used to heat columns have a large thermal mass and require more time to cool than a column that is resistively heated. The environmental temperature also affects the speed at which an oven and column can be cooled. The LTM-RHT column assemblies have an external fan mounted to the column assembly. The average cool-down time for the GC ABO, in which the maximum temperature was 250 °C, was 7.12 minutes. The average cool-down time for the LTM-RHT column

assembly was less than 3.5 minutes. By using the LTM-RHT column assemblies at a fast temperature ramping rate, overall retention time was reduced by more than 75% and the column recycling time was reduced by 50%, without any significant loss in resolution.

Field Analysis Results of Nerve and Blister Agents

Figure (8) provides a GC-MS (EI) total ion chromatogram obtained from the sampling and analysis of a 4-agent mixture in the field. The results were used to qualitatively determine if an LTM-RHT column combined with a GC-MS could effectively separate and analyze a mixture of CWAs (e.g. GB, GD, GF, and HD), in a field setting. Using the temperature ramping methodology discussed for CWA field sampling and analysis in Chapter 3, all analytes eluted well under 2 minutes. Although there was a decrease in resolution between the diastereomer peaks of GD due to the fast ramping, resolution between analyte peaks was sufficient for field identification.

Figure 8:

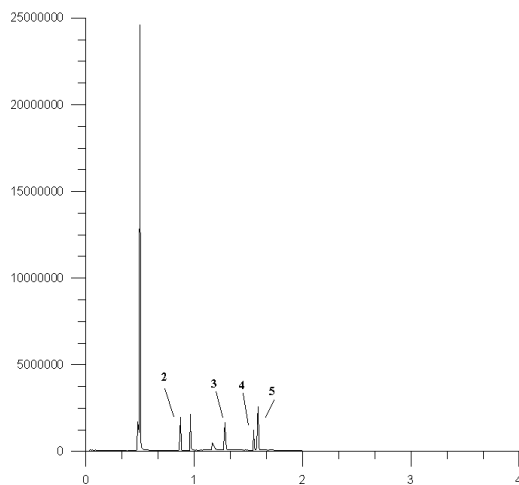


Figure 8: GC-MS chromatogram of chemical warfare agents using a Viking 572 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 5 min.) (Peaks: 2. Sarin, 3. Soman, 4. Sulfur Mustard, 5. Cyclohexyl methylphosphonofluoridate)

The results were used to qualitatively determine if an LTM-RHT column combined with a GC-MS could effectively separate and analyze a mixture of nerve agent precursors. In Figures 9 and 10, all analytes were analyzed and identified well under 2.44 and 1.26 minutes respectively. There was a decrease in resolution between peaks 3 and 4 as the LTM-RHT column temperature ramp rate was increased. However, the peaks were still sufficiently resolved to be able to identify their respective analytes. Faster temperature ramping may result in peaks 3 and 4 co-eluting. Fast temperature ramping may not be appropriate for very volatile compounds, because they may co-elute, resulting in masking of one or more of the analytes. However, if the chemicals being analyzed are known, a combination of fast temperature ramping and fast negative ramping maybe able to achieve rapid separation with co-elution.

Figure 9:

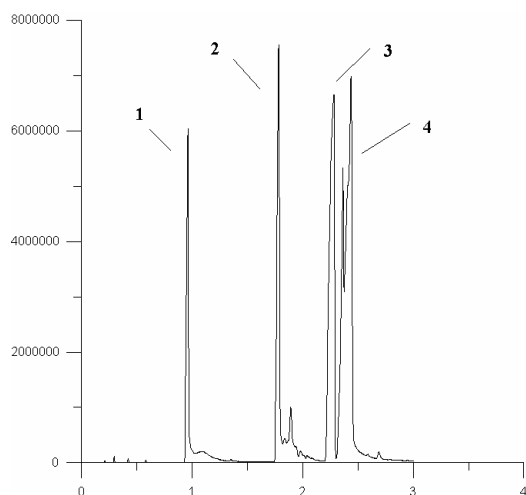


Figure 9: GC-MS chromatogram of chemical warfare agent precursors using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 sec. and temperature ramping rate of 40-250 at 20°C/min) (Peaks: 1. DMMP, 2. DEMP, 3. DIMP, 4. DEEP)

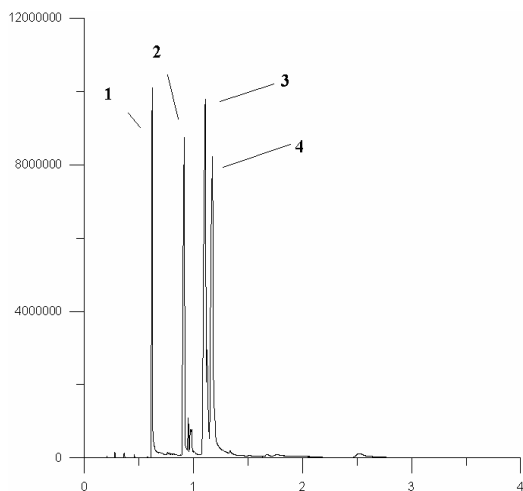
Figure 10:

Figure 10: GC-MS chromatogram of chemical warfare agent precursors using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 sec. and temperature ramping rate of 40-250 at 80°C/min) (Peaks: 1. DMMP, 2. DEMP, 3. DIMP, 4. DEEP)

Laboratory Analysis Results of Narcotics

Figures 11 and 12 show chromatograms for the five-narcotic mixture in which the LTM-RHT column was heated from 40°C to 250°C at 20°C per minute, and 40°C to 250°C at 60°C per minute, respectively. Figure 13 shows an example of a chromatogram for the five-narcotic mixture in which the LTM-RHT column was heated from 40°C to 250°C at 120°C per minute. The results demonstrate that FGC can significantly reduce analysis retention time while maintaining adequate peak resolution, and column efficiency. However, at ever increasing temperature ramping speeds, there is a decrease in the quality of the peak shape.

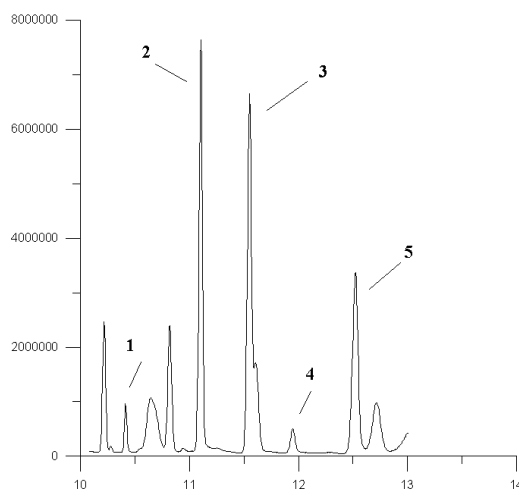
Figure 11:

Figure 11: GC-MS chromatogram of 5 µg/mL narcotic mixture using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1min. and temperature ramping rate of 40-250 at 20°C/min)(Peaks: 1. Remifentanyl, 2. Fentanyl, 3. Sufentanyl, 4. Carfentanyl, 5. Alfentanyl)

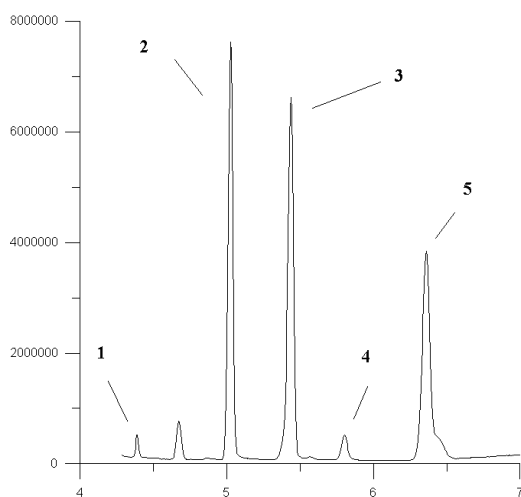
Figure 12:

Figure 12: GC-MS chromatogram of 5 µg/mL narcotic mixture using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber.(Sampling time: 1min. and temperature ramping rate of 40-250 at 60°C/min)(Peaks: 1. Remifentanyl, 2. Fentanyl, 3. Sufentanyl, 4. Carfentanyl, 5. Alfentanyl)

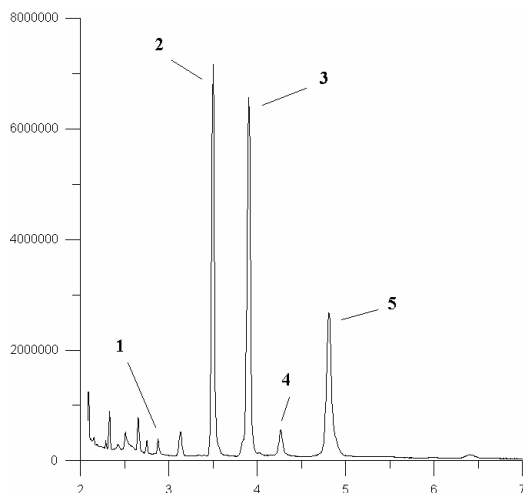
Figure 13:

Figure 13: GC-MS chromatogram of 5 µg/mL narcotic mixture using an Agilent 6890 GC-MS and RVM LTM-RHT DB1-MS column and a PDMS-DVB SPME sampling fiber. (Sampling time: 1 min. and temperature ramping rate of 40-250 at 120°C/min) (Peaks: 1. Remifentanyl, 2. Fentanyl, 3. Sufentanyl, 4. Carfentanyl, 5. Alfentanyl)

Numerous attempts were made to obtain consistent peak shape and resolution using the 120°C per minute temperature ramping rate, in order to compare the retention time (RT), column efficiency (CE), and peak resolution (PR) of this ramping rate, with that of the other two rates. Although additional runs were conducted using the former temperature ramping rate, consistent peak area and shape results could not be obtained. Often the peaks for Remifentanyl and Carfentanyl were flat. It may be possible to improve the resolution and shape of the peaks by using different carrier gas, such as Hydrogen, which doesn't lose efficiency as rapidly as Helium or Nitrogen. Manipulating the carrier gas pressure (i.e. pressure ramping) in conjunction with the temperature ramping method may also improve resolution and peak shape. Although the analytes' retention times and peak resolutions obtained using the 120°C per minute rate can not be quantitatively compared to the 20°C per minute and 60°C per minute rates, qualitatively the peaks were still sufficiently resolved to be able to identify their respective analytes.

Tables 4, 5 and 6 show RT, CE, and PR data obtained for each of the 5 narcotics that were analyzed using the two different temperature ramping rates. The results indicated that the RT, CE and PR for the analytes, using the faster ramping rate, were less than those obtained using the faster ramping rate. However, the optimum peak resolution was still maintained. The t-test showed that differences existed between the RTs ($p < 0.00$), CEs ($p < 0.02$), and PRs ($p < 0.017$) obtained using slow and fast temperature ramping rates. The null hypothesis stating that the RT, CE, and PR of analytes, obtained with a temperature ramping rate of 20°C per minute, is less than or equal to the RT, CE, and PR obtained at a rate of 60°C per minute is therefore rejected.

Table 4: Retention Time Comparative Analysis

	Ramping rate of 40 to 250 at 20°C				
Analytes	Remifentanil	Fentanyl	Sufentanil	Carfentanil	Alfentanil
Mean RT	10.411	11.102	11.548	11.942	12.523
STD DEV	0.003	0.002	0.005	0.002	0.008
	Ramping rate of 40 to 250 at 60°C				
Analytes	Remifentanil	Fentanyl	Sufentanil	Carfentanil	Alfentanil
Mean RT	4.371	5.025	5.436	5.800	6.355
STD DEV	0.038	0.007	0.007	0.007	0.009
p value (1-tailed, equal variances not assumed)	0.000	0.000	0.000	0.000	0.000

Retention Time (RT) – Amount of time from injection of analyte until the recording of its maximum peak

Table 5: Column Efficiency Comparative Analysis

	Ramping rate of 40 to 250 at 20°C				
Analytes	Remifentanil	Fentanyl	Sufentanil	Carfentanil	Alfentanil
Mean CE	45007.510	24372.807	53203.636	29316.227	20304.709
STD DEV	5183.032	2597.506	3210.606	3736.675	808.314
	Ramping rate of 40 to 250 at 60°C				
Analytes	Remifentanil	Fentanyl	Sufentanil	Carfentanil	Alfentanil
Mean CE	35775.496	5635.180	4137.973	6000.423	3891.374
STD DEV	6521.789	997.833	628.938	1592.813	2416.181
p value (1-tailed, equal variances not assumed)	0.020	0.000	0.000	0.000	0.000

Column Efficiency (CE) – Measured by column plate number, and related to peak sharpness and column performance

Table 6: Peak Resolution Comparative Analysis

	Ramping rate of 40 to 250 at 20°C			
Analyte Peaks	R - F	F - S	S - C	C - A
Mean PR	2.866	1.836	1.641	1.838
STD DEV	0.037	0.059	0.056	0.048
	Ramping rate of 40 to 250 at 60°C			
Analyte Peaks	R - F	F - S	S - C	C - A
Mean PR	3.609	1.352	1.133	1.491
STD DEV	0.290	0.064	0.073	0.248
P value (1-tailed, equal variances not assumed)	0.002	0.000	0.000	0.017

R – Remifentanil, F – Fentanyl, S – Sufentanil, C – Carfentanil, A – Alfentanil

Peak Resolution (PR) – Separation of two peaks in terms of their average peak width

CHAPTER FIVE: CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

Conclusions

The results of the laboratory sampling and analysis of the nerve and blister agents indicate that the LTM-RHT column assemblies could be used to effectively analyze and detect frank chemical warfare agent contaminated clothing 75% faster than a column that is heated using a standard ramping rate for an ABO. Depending on the characteristics of the analyte, such as volatility, the use of a LTH-RHT column assembly heated at a much faster rate than an ABO heated column will allow for a significant increase in the number of samples that can be analyzed within a given time period.

The CWA field sampling and analysis results indicate that the LTM-RHT column will significantly reduce over all analysis time, including column cool down time between runs. The system can be used by an ERU to rapidly analyze and identify CWAs without a significant loss in peak resolution. Reducing the analysis time will benefit ERUs by rapidly providing qualitative identification of chemicals directly to the on-scene command center [1, 18, 50].

The analysis results of the field simulated CWA precursors show that identification along with sufficient peak separation of known volatile analytes, via FGC, is obtainable. The ability to rapidly detect CWA precursors in the field will assist weapons inspectors in locating possible manufacturing sites.

The qualitative and quantitative comparative FGC analysis of the narcotic mixture indicates that retention time can be significantly reduced while maintaining adequate peak resolution and column efficiency.

When responding to an emergency related to inadvertent or intentional release of chemicals, rapid identification of the chemical hazards present is essential. Rapid identification provides invaluable information when making decisions regarding protection of the response personnel, general public, and the environment. It also provides medical personnel with information useful in the treatment of casualties. This work has demonstrated that FGC coupled with mass spectral detection can rapidly provide ERUs with this vital information in a field environment. While the rapid separation and identification was achieved for CWAs, CWA precursors and non-traditional CWAs in the form of narcotics, the described sampling and analysis method has potential application for any event where rapid on-site identification of volatile and semi-volatile organic compounds is desired.

Limitations and Recommendations

This research only involved the sampling and analysis of known agents predominantly under laboratory conditions. When rapidly ramping the column to a high temperature, one chemical may mask another because they have co-eluted from the column. For unknown sampling in the field, it is recommended that a lower temperature ramping be performed in addition or prior to the fast ramp to check for co-elution. To improve peak shape and resolution, short sampling times using SPME, should be performed to reduce the affect of column loading if contaminant concentrations are expected to be high. High temperature ramping rates can result in poor peak shape. Additional research should be performed to determine if other variables such as carrier gas flow rate, analyte concentration, column loading, and pressure ramping could be modified to improve peak shape and resolution while ramping the temperature of the

LTM-RHT column.

In the field, CWAs or narcotics may be found mixed with organic material such as soil or body fluids (i.e. blood and urine). This study does not indicate if CWA contaminated soil or body fluids can be sampled and effectively analyzed using FGC. Nor does the study indicate the minimum level of detection at which the analytes can be analyzed. The research does not provide information on how the characteristics of the equipment, such as column bleed and background levels will affect the potential for the equipment to analyze trace levels of chemicals (i.e. nanograms or lower). Further testing is recommended to determine what effects other chemicals and common environmental matrices, may have on the analytical systems ability to separate and identify the desired analytes. Additional research should be performed to determine if the rapid heating and cooling increases the natural bleed from the column potentially reducing the ability to detect analytes at trace concentrations.

The ability to use SPME and LTM-RHT columns to evaluate personal exposure to chemicals using biological field samples (i.e. saliva, and urine) was not evaluated. Research, involving the use of human or live animal models could provide information on the effectiveness of SPME in sampling for chemicals in the field. Such information would invaluable for law enforcement, crime scene investigators, and forensic specialist [52].

Finally, to improve the portability of the equipment, and make it rapidly fieldable by an ERU, the ABO of the GC could be totally removed and replaced directly with the LTM-RHT column assembly.

BIBLIOGRAPHY

1. CBIRF, www.globalsecurity.org/military/arency/usmc/cbirf.htm. 2004.
2. Division, C.C.C., *Medical Management of Chemical Casualties Handbook*. 1999: Chemical Casualty Care Division.
3. CBWNP, *CNS The Moscow theater hostage crisis: incapacitating and chemical warfare*. 2003, Monterey institute of international studies.
4. Sun, Y. and K.Y. Ong, *Detection Technologies for Chemical Warfare Agents and Toxic Vapors*. 2005, Washington D.C.: CRC Press.
5. Hook, G.L., C. Jackson-Lepage, S.I. Miller, and P.A. Smith, *Dynamic Solid Phase Microextraction for Sampling of Airborne Sarin with Gas Chromatography-Mass Spectrometry for Rapid Field Detection and Quantification*. *Journal of Separation Science*, 2004. 27: p. 1017-1022.
6. Hook, G.L., G.L. Kimm, T. Hall, and P.A. Smith, *Solid-phase microextraction (SPME) for rapid field sampling and analysis by gas chromatography-mass spectrometry (GC-MS)*. *Trends in Analytical Chemistry*, 2002. 21(8): p. 534-542.
7. Smith, P.A. and S. MacDonald, *Gas chromatography using resistively heated column with mass spectrometric detection for rapid analysis of pyridine released from Bacillus spores*. *Journal of Chromatography A*, 2004. 1036: p. 249-253.
8. Smith, P.A., C. Jackson-Lepage, D.R. Koch, H. Wyatt, B.A. Eckenrode, G.L. Hook, G. Betsinger, and R. Erickson, *Detection of gas phase chemical warfare agents using field-portable chromatography-mass spectrometry systems: instrument and sampling strategy considerations*. *Trends in Analytical Chemistry*, 2004. 23(4): p. 296-306.
9. Muller, L., *Field Analysis by SPME*, in *Applications of Solid Phase Microextraction*, J. Pawliszyn, Editor. 1999, Royal Society of Chemistry: Herefordshire UK.
10. Gorecki, T. and J. Pawliszn, *Field of Analytical Chemistry and Technology*, 1997. 1(5): p. 227

11. Overton, H.B., H.P. Dharmasena, U. Ehrmann, and K.R. Carnay, *Trends and advances in portable analytical instrumentation*. Field Analytical Chemistry and Technology, 1996. 1(2): p. 87.
12. Smith, P.A., M.V. Sheely, and T.A. Kluchinsky, Jr., *Solid phase microextraction with analysis by gas chromatography to determine short term hydrogen cyanide in a field setting*. Journal of Separation Science, 2002. 25: p. 917.
13. Sng, M.T. and W.F. Ng, *In-situ derivatization of degradation products of chemical warfare agents in water by solid-phase microextraction and gas chromatographic-mass spectrometric analysis*. Journal of Chromatography A, 1999. 832: p. 173.
14. Szostek, B. and J.H. Aldestadt, *Determination of organoarsenicals in the environment by solid-phase microextraction-gas chromatography-mass spectrometry*. Journal of Chromatography A, 1998. 807: p. 253.
15. Smith, P.A., T.A. Kluchinsky, Jr., P.B. Savage, R.P. Erickson, A.P. Lee, K. Williams, M. Stevens, and R.J. Thomas, *Traditional Sampling with Laboratory Analysis and Solid Phase Microextraction Sampling with Field Gas Chromatography/Mass Spectrometry by Military Industrial Hygienists*. AIHA Journal, 2002. 62: p. 284-292.
16. Kluchinsky, T.A., Jr., M.V. Sheely, P.B. Savage, and P.A. Smith, *Formation of 2-chlorobenzydenemalononitrile (CS riot control agent) thermal degradation products at elevated temperatures*. Journal of Chromatography A, 2002. 952: p. 205.
17. Black, R.M., R.J. Clarke, D.B. Cooper, R.W. Read, and D. Utley, *Application of headspace analysis, solvent extraction, thermal desorption and gas chromatography-mass spectrometry to the analysis of chemical warfare samples containing sulphur mustard and related compounds*. Journal of Chromatography, 1993. 637: p. 71.
18. Santos, F.J. and M.T. Galceran, *Modern developments in gas chromatography-mass spectrometry-based environmental analysis*. Journal of Chromatography A, 2003. 1: p. 1.
19. Hook, G.L., G.L. Kimm, D.R. Koch, P.B. Savage, B. Ding, and P.A. Smith, *Detection of VX Contamination in Soil through Solid Phase Micro-extraction Sampling and Gas Chromatography/Mass Spectrometry of the VX Degradation Product Bis(diisopropylaminoethyl)disulfide*. Journal of Chromatography A, 2003. 992: p. 1-9.

20. Sloan, K.M., R.V. Mustacich, and B.A. Eckenrode, *Development and Evaluation of a Low Thermal Mass Gas Chromatograph for Rapid Forensic GC-MS Analysis*. Field Analytical Chemistry and Technology, 2001. 5(6): p. 288-301.
21. *Implementation and application of joint medical surveillance for deployments*, in *DOD Instruction 6490.3*. 1997, Department of Defense.
22. Gorecki, T. and J. Pawliszyn, Field Analytical Chemistry and Technology, 1997. 1(5): p. 227.
23. Muller, L., *Applications of Solid Phase Microextraction*. J Pawliszyn ed, ed. J. Pawliszyn. 1999, Herfordshire UK: Royal Society of Chemistry.
24. Overton, H.B., H.P. Dharmasena, U. Ehrmann, and K.R. Carney, Field Analytical Chemistry Technology, 1996. 1(2): p. 87.
25. Council, N.S.a.T., *A National Obligation: Planning for Health Preparedness for and readjustments of the Military, Veterans, and Their Families after Future Deployments*. 1998.
26. Langford, R.E., *Introduction to Weapons of Mass destruction Radiological, Chemical, and Biological*. 2004, New Jersey: John Wiley and Sons Inc.
27. Ellison, D.H., *Handbook of Chemical and Biological Warfare Agents*. 1999, Washington D.C.: CRC Press.
28. Munro, N., S.S. Talmage, G.D. Griffin, L.C. Walters, A.P. Watson, J.F. King, and V. Hauschild, *The Sources, Fate, and Toxicity of Chemical Warfare Agent Degradation Products*. Environmental Health Perspectives, 1999. 107(12): p. 933-974.
29. Wax, P.A., C.E. Becker, and S.C. Curry, *Unexpected "Gas" Casualties in Moscow: A Medical Toxicology Perspective*. Annals of Emergency Medicine, 2003. 41(5): p. 700-705.
30. Pike, J., *Chemical Warfare Agents*. 2003.
31. USAMRICD, *Field Management of Chemical Casualties Handbook*. 2003, U.S. Army Medical Research Institute of Chemical Defense.

32. <http://WWW.cbwinfo.com/chemical/nerve/GF.shtml>, *GF*. 2003, CBWINFO.
33. Goodman, G., *The Pharmacological Basis of Therapeutics*. 10 ed. 2001, New York: McGraw-Hill.
34. <http://hazard.com/msds/tox/f/q99/q46.html>, *Opioids*. 2003, Hazard.Com.
35. <http://chemfinder.cambridgesoft.com/result.asp>, *Opioids*. 2003, Chemfinder.
36. Pharmaceutica, J., *MSDS*. 2003, Janssen Pharmaceutica.
37. Hooijschuur, E.W.J., C.E. Kientz, and U.A.T. Brinkman, *Analytical separation techniques for the determination of chemical warfare agents*. *Journal of Chromatography A*, 2002. 982: p. 177-200.
38. Carrick, W., D.B. Cooper, and B. Muir, *Retrospective identification of chemical warfare agents by high-temperature automatic thermal desorption-gas chromatography-mass spectrometry*. *Journal of Chromatography A*, 2001. 925: p. 241-249.
39. Hook, G.L., G.L. Kimm, G. Betsinger, P.B. Savage, A. Swift, T. Logan, and P.A. Smith, *Solid phase microextraction sampling and gas chromatography/mass spectrometry for field detection of the chemical warfare agent O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX)*. *Journal of Separation Science*, 2003. 26: p. 1091.
40. Kimm, G.L., G.L. Hook, and P.A. Smith, *Application of headspace solid-phase microextraction and gas chromatography-mass spectrometry for detection of the chemical warfare agent bis(2-chloroethyl) sulfide in soil*. *Journal of Chromatography A*, 2002. 971(185).
41. Lakso, H.A. and W.F. Ng, *Determination of Chemical Warfare Agents in Natural water Samples by Solid-Phase Microextraction*. *Analytical Chemistry*, 1997. 69: p. 1866-1872.
42. Pawliszyn, J., *Solid Phase Microextraction Theory and Application*. 1997, New York: Wiley-VCH.

43. Editor, *Solid Phase Microextraction Theory and Optimization*. Supelco Bulletin, 1998.
44. Matisova, E. and M. Domotorova, *Fast gas chromatography and its use in trace analysis*. Journal of Chromatography A, 2003. 1: p. 1.
45. Schneider, J.F., A.S. Boparai, and L.L. Reed, *Screening for Sarin in air and water by solid-phase microextraction-Gas Chromatography-Mass Spectrometry*. Journal of Chromatographic Science, 2001. 39: p. 420.
46. Dalluge, J., R.J.J. Vreuls, U.A.T. Brinkman, and R. Ou-Aissa, *Fast Temperature Programming in Gas Chromatography using Resistive Heating*. Journal of High Resolution Chromatography, 1999. 22(8): p. 459-464.
47. Smith, P.A. and M. Stephen, *Use of Flash Gas Chromatography with Mass Spectrometry for Rapid Analysis of Pyridine Released from Biological Material Using a Furnace Inlet System*. Draft, Submitted.
48. Dalluge, J., R.J.J. Vreuls, D.J. van Iperen, M. van Rijn, and U.A.T. Brinkman, *Resistively heated gas chromatography coupled to quadrupole mass spectrometry*. Journal of Separation Science, 2002. 25: p. 608-614.
49. van Deursen, M., J. Beens, C.A. Cramers, and H.-G. Janssen, *Possibilities and Limitations of Fast Temperature Programming Route towards Fast GC*. Journal of High Resolution Chromatography, 1999. 22(9): p. 509.
50. Mastovska, K. and S.J. Lehotay, *Practical approaches to fast gas chromatography-mass spectrometry*. Journal of Chromatography A, 2003. 1: p. 1.
51. David, F., D.R. Gere, F. Scanlon, and P. Sandra, *Instrumentation and applications of fast high-resolution capillary gas chromatography*. Journal of Chromatography A, 1999. 842: p. 309.
52. Williams, T., M. Riddle, S.L. Morgan, and W.E. Brewer, *Rapid Gas Chromatographic Analysis of Drugs of Forensic Interest*. Journal of Chromatographic Science, 1999. 37: p. 210-214.

53. van Lieshout, M., R. Derks, H.-G. Janssen, and C.A. Cramers, *Fast Capillary Gas Chromatography: Comparison of Different Approaches*. Journal of High Resolution Chromatography, 1998. 21(11): p. 583-586.
54. Hail, M.E. and R.A. Yost, *Compact Gas Chromatograph Probe for Gas Chromatography/Mass Spectrometry Utilizing Resistively Heated Aluminum-Clad Capillary Columns*. Analytical Chemistry, 1989. 61: p. 2410-2418.
55. McNair, H.M. and G.L. Reed, *Fast Gas Chromatography: The Effect of Fast Temperature Programming*. 2000.
56. http://whatis.techtarget.com/definition/0,sid9_gci214066,00.html, *Toroid*. 2005, whatis.techtarget.com.
57. Hook, G., C. Jackson-Lepage, S.I. Miller, and P.A. Smith, *Dynamic SPME for sampling airborne Sarin with GC-MS for rapid field detection and quantification*. 2003.
58. Smith, P.A., C. Jackson-Lepage, D. Koch, H. Wyatt, B.A. Eckenrode, G.L. Hook, and H. Betsinger, *Detection of Gas Phase Chemical Warfare Agents using Field-Portable Gas Chromatography-Mass Spectrometry Systems: Instrument and Sampling Strategy Considerations*. 2003.
59. Paradis, C., C. Dufresne, M. Bolon, and R. Boulieu, *Solid-Phase Microextraction of Human Plasma Samples for Determination of Sufentanil by Gas Chromatography-Mass Spectrometry*. Therapeutic Drug Monitor, 2002. 24(6): p. 768-774.
60. Rood, D., *A practical guide to the care, maintenance, and trouble shooting of capillary gas chromatographic systems*. 3rd revised ed. ed. 1999, New York: Wiley-VCH.
61. Grob, R.L., *Modern Practice of Gas Chromatography*. 3rd ed ed. 1995, New York: John Wiley and Sons inc.
62. Dams, R., W.E. Lambert, K.M. Clauwaert, and A.P. DeLeenheer, *Comparison of phenyl-type columns in the development of a fast liquid chromatographic system for eighteen opiates commonly found in forensic toxicology*. Journal of Chromatography A, 2000. 896: p. 311-319.